8

cagctacatg ccattaatct ggaaggaacg ggcaggaaag ccaccatgca aacaacccag
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ggagagatca acttcggagg gtctgggaag aagcgaggca agtttgtgaa ggtgccaagc
agtgtggccc cctctgtgct ttttgaactc ctgctcaccg agtggcacct gccagccccc
aacctggtgg tgtccctggt gggtgaggaa cgacctttgg ctatgaagtc gtggcttcgg
gatgtcctgc gcaaggggct ggtgaaagca gctcagagca caggtgcctg gatcctgacc
agtgccctcc acgtgggcct ggcccgccat gttggacaag ctgtacgtga tcactctctg
gctagcacat ccaccaagat ccgtgtagtg gccatcggaa tggcctctct ggatcgaatc
cttcaccgtc aacttctaga tggtgtccac caaaaggagg atactcccat ccactaccca
gcagatgagg gcaacattca gggacccctc tgccccctgg acagcaatct ctcccacttc
atccttgtgg agtcaggcgc ccttgggagt gggaacgacg ggctgacaga gctgcagctg
agcctggaga agcacatctc tcagcagagg acaggttatg ggggcaccag ctgcatccag
atacctgtcc tttgcctgtt ggtcaatggt gaccccaaca ccctagagag gatttccagg
gcagtggagc aggctgcccc atggctgatc ctggcaggtt ctggtggcat tgctgatgta
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caagaagccc aagactacct agatgagctc aagttagcag tggcctggga tcgcgtggac
attgccaaga gtgaaatctt caatggggac gtggaatgga agtcctgtga cttggaagag
gtgatgacag atgccctcgt gagcaacaag cctgactttg tccgcctctt tgtggacagc
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ctggccggcc tgggtgccca gcaggctcgg gagctgccca ttggtctgcc tgccttctca
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ctgccagacc tcagtaggaa gagtgaagac ccttggaggg acctgttcct ctgggctgtg
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gacggtgtgc aagcattcct gaccaagatc tggtggggag acatggccac aggcacaccc
atcctacggc ttctgggtgc cttcacctgc ccagccctca tctacacaaa cctcatctcc
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gtgccctcgg tgtttgaggc tggcaggacc gttctggcca ttgacttcat ggtgttcaca
cttcggctca tccacatctt tgctattcac aagcagttgg gtcctaagat catcattgta
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cgccgtgtgc tatacaggcc ttacctgcag atctttgggc aaatccctct ggatgaaatt
gatgaggctc gtgtgaactg ttctcttcac cctctgctgc tggaaagctc ggcttcctgc
cctaatctct atgccaactg gctggtcatt ctcctgctgg ttaccttcct gcttgtcact

FIG.1A

aatgtgctgc tcatgaacct tctgatcgcc atgttcagct acacattcca ggtggtgcaa
ggcaatgcag acatgttctg gaagtttcaa cgctaccacc tcatcgttga ataccatgga
agaccagctc tggccccgcc cttcatcctg ctcagccacc tgagcctggt gctcaagcag
gtcttcagga aggaagccca gcataagcga caacatctgg agagagactt gcctgacccc
ttggaccaga agatcattac ctgggaaacg gttcaaaagg agaacttcct gagtaccatg
gagaaacgga ggagggacag cgagggggag gtgctgagga aaacggcaca cagagtggac
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tcacaggcca actactgtat gctcctcttg tcctctatga cggatacact ggctccagga
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cctaagagaa tgaaactcat gtctttggca tctattcggg agcctcagaa gtatcctctc
cagcagggca agatttttca tgtcccacta aagctttcac tggcttggac tggacagctg
gatctggcca agtcctacat aggacaccat ctgcctggat ggggctattt aggtctaacc
cctgtcttac cctgagttcc taagaagcca acctcttaaa cactaggttt ctttctgacc
cctgacccac tcattagctg accagctcct agagggcagg actcagatct attgtaatta
cctcccatct ttcacccccc acagcattat ctgtctgatc attctggcag aaaccccaag
atattgctca agggtaccca atgctacttt actttctata aagcctgtag accacctcaa
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
aaaaaaaaaa aaaaaaa

FIG.1B

MQTTQSSCPGSPPDTEDGWEPILCRGEINFGGSGKKRGKFVKVPSSVAPSVLFELLLTEWHLPAPNLVVSLVGEERPLAMKSWLRDVLR
KGLVKAAQSTGAWILTSALHVGLARHVGQAVRDHSLASTSTKIRVVAIGMASLDRILHRQLLDGVHQKEDTPIHYPADEGNIQGPLCPL
DSNLSHFILVESGALGSGNDGLTELQLSLEKHISQQRTGYGGTSCIQIPVLCLLVNGDPNTLERISRAVEQAAPWLILAGSGGIADVLA
ALVSQPHLLVPQVAEKQFREKFPSECFSWEAIVHWTELLQNIAAHPHLLTVYDFEQEGSEDLDTVILKALVKACKSHSQEAQDYLDELK
LAVAWDRVDIAKSEIFNGDVEWKSCDLEEVMTDALVSNKPDFVRLFVDSGADMAEFLTYGRLQQLYHSVSPKSLLFELLQRKHEEGRLT
LAGLGAQQARELPIGLPAFSLHVSRVLKDFLHDACRGFYQDGRRMEERGPPKRPAGQKWLPDLSRKSEDPWRDLFLWAVLQNRYEMATY
FWAMGREGVAAALAACKIIKEMSHLEKEAEVARTMREAKYEQLALDLFSECYGNSEDRAFALLVRRNHSWSRTTCLHLATEADAKAFFA
HDGVQAFLTKIWWGDMATGTPILRLLGAFTCPALIYTNLISFSEDAPQRMDLEDLQEPDSLDMEKSFLCSRGGQLEKLTEAPRAPGDLG
PQAAFLLTRWRKFWGAPVTVFLGNVVMYFAFLFLFTYVLLVDFRPPPQGPSGSEVTLYFWVFTLVLEEIRQGFFTDEDTHLVKKFTLYV
EDNWNKCDMVAIFLFIVGVTCRMVPSVFEAGRTVLAIDFMVFTLRLIHIFAIHKQLGPKIIIVERMMKDVFFFLFFLSVWLVAYGVTTQ
ALLHPHDGRLEWIFRRVLYRPYLQIFGQIPLDEIDEARVNCSLHPLLLESSASCPNLYANWLVILLLVTFLLVTNVLLMNLLIAMFSYT
FQVVQGNADMFWKFQRYHLIVEYHGRPALAPPFILLSHLSLVLKQVFRKEAQHKRQHLERDLPDPLDQKIITWETVQKENFLSTMEKRR
RDSEGEVLRKTAHRVDLIAKYIGGLREQEKRIKCLESQANYCMLLLSSMTDTLAPGGTYSSSQNCGCRSQPASARDREYLESGLPPSDT

FIG.2

atgcaggatg tccaaggccc ccgtcccgga agccccgggg atgctgaaga ccggcgggag
ctgggcttgc acaggggcga ggtcaacttt ggagggtctg ggaagaagcg aggcaagttt
gtacgggtgc cgagcggagt ggccccgtct gtgctctttg acctgctgct tgctgagtgg
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aagtcctggc tgcgggatgt gctgcgcaag gggctggtga aggcggctca gagcacagga
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cgcgaccact cgctggccag cacgtccacc aaggtccgtg tggttgctgt cggcatggcc
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tcccacttca tcctggtgga gccaggcccc ccggggaagg gcgatgggct gacggagctg
cggctgaggc tggagaagca catctcggag cagagggcgg gctacggggg cactggcagc
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gatgtgcttg ctgccctagt gaaccagccc cacctcctgg tgcccaaggt ggccgagaag
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aaggccttct ttgcccacga cggcgttcag gccttcctga ccaggatctg gtgggggggac
atggccgcag gcacgcccat cctgcggctg ctaggagcct tcctctgccc cgccctcgtc
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atgtacttcg ccttcctctt cctgttcacc tacgtcctgc tggtggactt caggccgccc
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gaggactcac catcctgccc cagcctctat gccaactggc tggtcatcct cctgctggtc
accttcctgt tggtcaccaa tgtgctgctc atgaacctgc tcatcgccat gttcagctac
acgttccagg tggtgcaggg caacgcagac atgttctgga agttccagcg ctacaacctg

FIG.3A

```
attgtggagt accacgagcg ccccgccctg gccccgccct tcatcctgct cagccacctg
agcctgacgc tccgccgggt cttcaagaag gaggctgagc acaagcggga gcacctggag
agagacctgc cagacccccc ggaccagaag gtcgtcacct gggagacagt ccagaaggag
aacttcctga gcaagatgga gaagcggagg agggacagcg agggggaggt gctgcggaaa
accgcccaca gagtggactt cattgccaag tacctcgggg ggctgagaga gcaagaaaag
cgcatcaagt gtctggagtc acagatcaac tactgctcgg tgctcgtgtc ctccgtggct
gacgtgctgg cccagggtgg cggcccccgg agctctcagc actgtggcga gggaagccag
ctggtggctg ctgaccacag aggtggttta gatggctggg aacaacccgg ggctggccag
cctccctcgg acacatga
```

FIG.3B

```
MQDVQGPRPG SPGDAEDRRE LGLHRGEVNF GGSGKKRGKF VRVPSGVAPS
VLFDLLLAEW HLPAPNLVVS LVGEEQPFAM KSWLRDVLRK GLVKAAQSTG
AWILTSALRV GLARHVGQAV RDHSLASTST KVRVVAVGMA SLGRVLHRRI
LEEAQEDFPV HYPEDDGGSQ GPLCSLDSNL SHFILVEPGP PGKGDGLTEL
RLRLEKHISE QRAGYGGTGS IEIPVLCLLV NGDPNTLERI SRAVEQAAPW
LILVGSGGIA DVLAALVNQP HLLVPKVAEK QFKEKFPSKH FSWEDIVRWT
KLLQNITSHQ HLLTVYDFEQ EGSEELDTVI LKALVKACKS HSQEPQDYLD
ELKLAVAWDR VDIAKSEIFN GDVEWKSCDL EEVMVDALVS NKPEFVRLFV
DNGADVADFL TYGRLQELYR SVSRKSLLFD LLQRKQEEAR LTLAGLGTQQ
AREPPAGPPA FSLHEVSRVL KDFLQDACRG FYQDGRPGDR RRAEKGPAKR
PTGQKWLLDL NQKSENPWRD LFLWAVLQNR HEMATYFWAM GQEGVAAALA
ACKILKEMSH LETEAEAARA TREAKYERLA LDLFSECYSN SEARAFALLV
RRNRCWSKTT CLHLATEADA KAFFAHDGVQ AFLTRIWWGD MAAGTPILRL
LGAFLCPALV YTNLITFSEE APLRTGLEDL QDLDSLDTEK SPLYGLQSRV
EELVEAPRAQ GDRGPRAVFL LTRWRKFWGA PVTVFLGNVV MYFAFLFLFT
YVLLVDFRPP PQGPSGPEVT LYFWVFTLVL EEIRQGFFTD EDTHLVKKFT
LYVGDNWNKC DMVAIFLFIV GVTCRMLPSA FEAGRTVLAM DFMVFTLRLI
HIFAIHKQLG PKIIVVERMM KDVFFFLFFL SVWLVAYGVT TQALLHPHDG
RLEWIFRRVL YRPYLQIFGQ IPLDEIDEAR VNCSTHPLLL EDSPSCPSLY
ANWLVILLLV TFLLVTNVLL MNLLIAMFSY TFQVVQGNAD MFWKFQRYNL
IVEYHERPAL APPFILLSHL SLTLRRVFKK EAEHKREHLE RDLPDPLDQK
VVTWETVQKE NFLSKMEKRR RDSEGEVLRK TAHRVDFIAK YLGGLREQEK
RIKCLESQIN YCSVLVSSVA DVLAQGGGPR SSQHCGEGSQ LVAADHRGGL
DGWEQPGAGQ PPSDT*
```

FIG.4

```
mTrp8   MQTTQSSCPGSPPDTEDGWEPILCRGEINFGGSGKKRGKFVKVPSSVAPSVLFELLLTEW 60
hTRP8   MQDVQGPRPGSPGDAEDRRELGLHRGEVNFGGSGKKRGKFVRVPSGVAPSVLFDLLLAEW 60
        ** .*.. **** *:**  *  * ***:**********:***.*******:***:**

mTrp8   HLPAPNLVVSLVGEERPLAMKSWLRDVLRKGLVKAAQSTGAWILTSALHVGLARHVGQAV 120
hTRP8   HLPAPNLVVSLVGEEQPFAMKSWLRDVLRKGLVKAAQSTGAWILTSALRVGLARHVGQAV 120
        ***************:*:*********************************:**********

mTrp8   RDHSLASTSTKIRVVAIGMASLDRILHRQLLDGVHQKEDTPIHYPADEGNIQGPLCPLDS 180
hTRP8   RDHSLASTSTKVRVVAVGMASLGRVLHRRILEEAQ--EDFPVHYPEDDGGSQGPLCSLDS 178
        ***********:****:*****.*:***::*: .:  ** *:*** *:*. *****.***

mTrp8   NLSHFILVESGALGSGNDGLTELQLSLEKHISQQRTGYGGTSCIQIPVLCLLVNGDPNTL 240
hTRP8   NLSHFILVEPGPPGKG-DGLTELRLRLEKHISEQRAGYGGTGSIEIPVLCLLVNGDPNTL 237
        *********.*. *.* ******:* ******:**:*****..*:***************

mTrp8   ERISRAVEQAAPWLILAGSGGIADVLAALVSQPHLLVPQVAEKQFREKFPSECFSWEAIV 300
hTRP8   ERISRAVEQAAPWLILVGSGGIADVLAALVNQPHLLVPKVAEKQFKEKFPSKHFSWEDIV 297
        ****************.*************.*******:******:*****: **** **

mTrp8   HWTELLQNIAAHPHLLTVYDFEQEGSEDLDTVILKALVKACKSHSQEAQDYLDELKLAVA 360
hTRP8   RWTKLLQNITSHQHLLTVYDFEQEGSEELDTVILKALVKACKSHSQEPQDYLDELKLAVA 357
        :**:*****::* **************:*******************.************

mTrp8   WDRVDIAKSEIFNGDVEWKSCDLEEVMTDALVSNKPDFVRLFVDSGADMAEFLTYGRLQQ 420
hTRP8   WDRVDIAKSEIFNGDVEWKSCDLEEVMVDALVSNKPEFVRLFVDNGADVADFLTYGRLQE 417
        ****************************.********:*******.***:*:*********:

mTrp8   LYHSVSPKSLLFELLQRKHEEGRLTLAGLGAQQARELPIGLPAFSLHEVSRVLKDFLHDA 480
hTRP8   LYRSVSRKSLLFDLLQRKQEEARLTLAGLGTQQAREPPAGPPAFSLHEVSRVLKDFLQDA 477
        **:*** *****:*****:**.********:***** * * ****************:**

mTrp8   CRGFYQDGR----RMEERGPPKRPAGQKWLPDLSRKSEDPWRDLFLWAVLQNRYEMATYF 536
hTRP8   CRGFYQDGRPGDRRRAEKGPAKRPTGQKWLLDLNQKSENPWRDLFLWAVLQNRHEMATYF 537
        *********    *  *:**.***:***** **.:***:**************:******

mTrp8   WAMGREGVAAALAACKIIKEMSHLEKEAEVARTMREAKYEQLALDLFSECYGNSEDRAFA 596
hTRP8   WAMGQEGVAAALAACKILKEMSHLETEAEAARATREAKYERLALDLFSECYSNSEARAFA 597
        ****:************:*******.***.**: ******:**********.*** ****
```

FIG.5

Classification and Secondary Structure Prediction of Membrane Proteins

http://azusa.proteome.bio.tuat.ac.jp/sosui/

Orientation of the N-terminus of	mTrp8:	IN		
Number of transmembrane helices of	mTrp8:	6		
Position of transmembrane helices of	mTrp8:	helix	begin	end
		1	732	754
		2	769	792
		3	807	829
		4	839	863
		5	870	893
		6	955	977

Orientation of the N-terminus of	hTrp8:	IN		
Number of transmembrane helices of	hTrp8:	6		
Position of transmembrane helices of	hTrp8:	helix	begin	end
		1	733	755
		2	770	792
		3	807	829
		4	843	863
		5	873	893
		6	955	977

FIG.6A

HYDROPHOBICITY PROFILE OF mTrp8 (MADE WITH DNAMAN SOFTWARE)

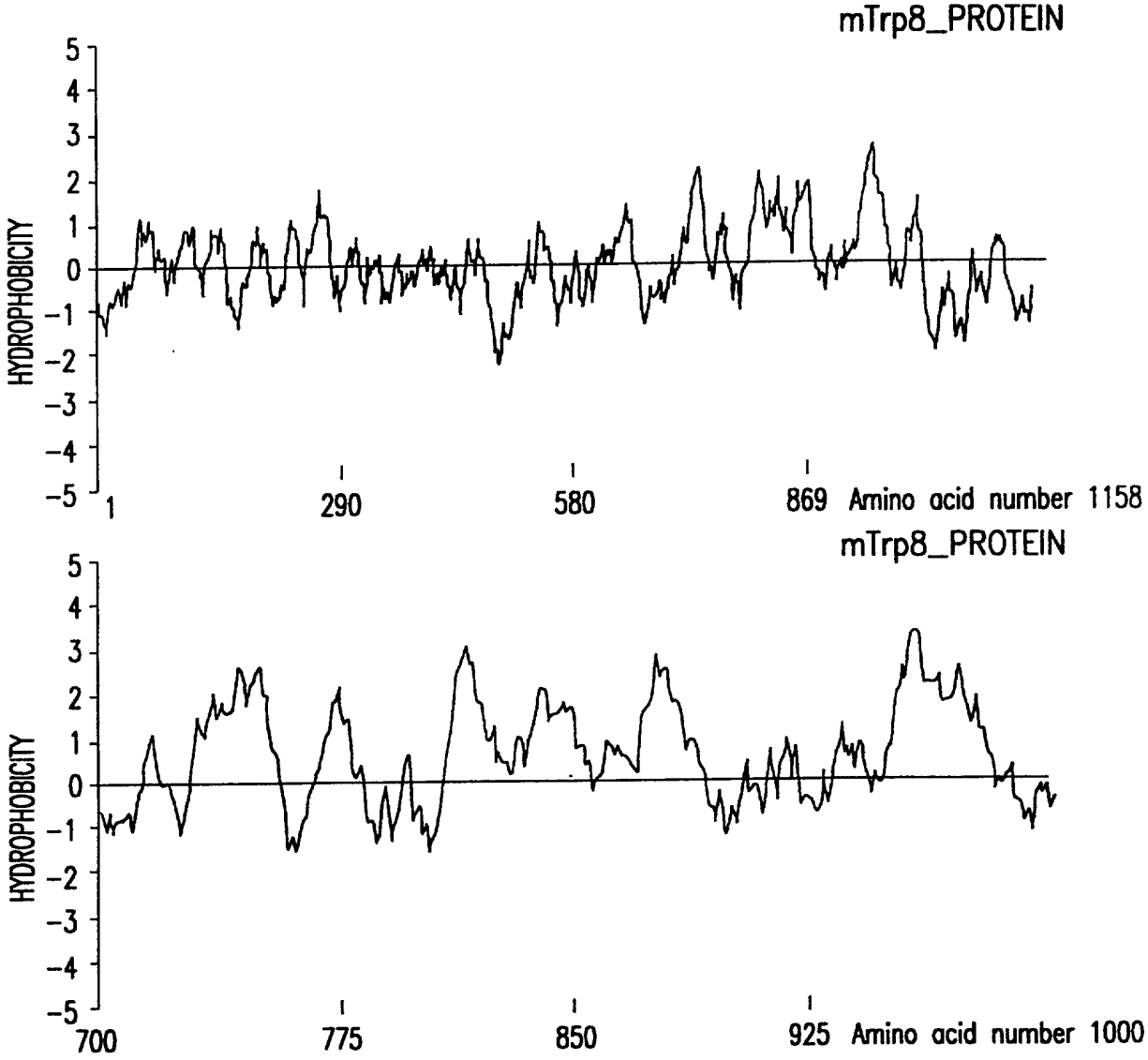

FIG. 6B

HYDROPHOBICITY PROFILE OF hTrp8 (MADE WITH DNAMAN SOFTWARE)

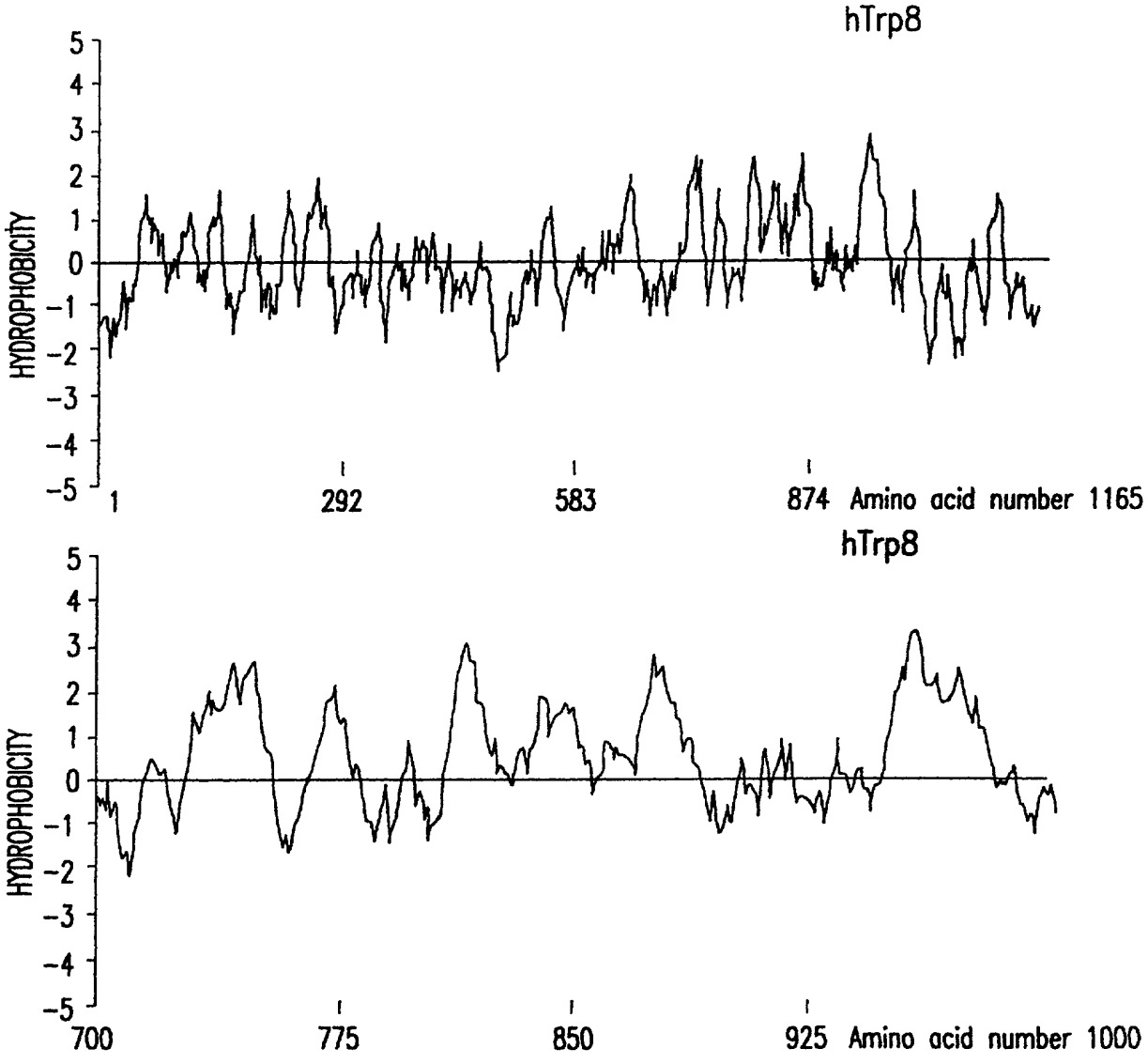

FIG. 6C

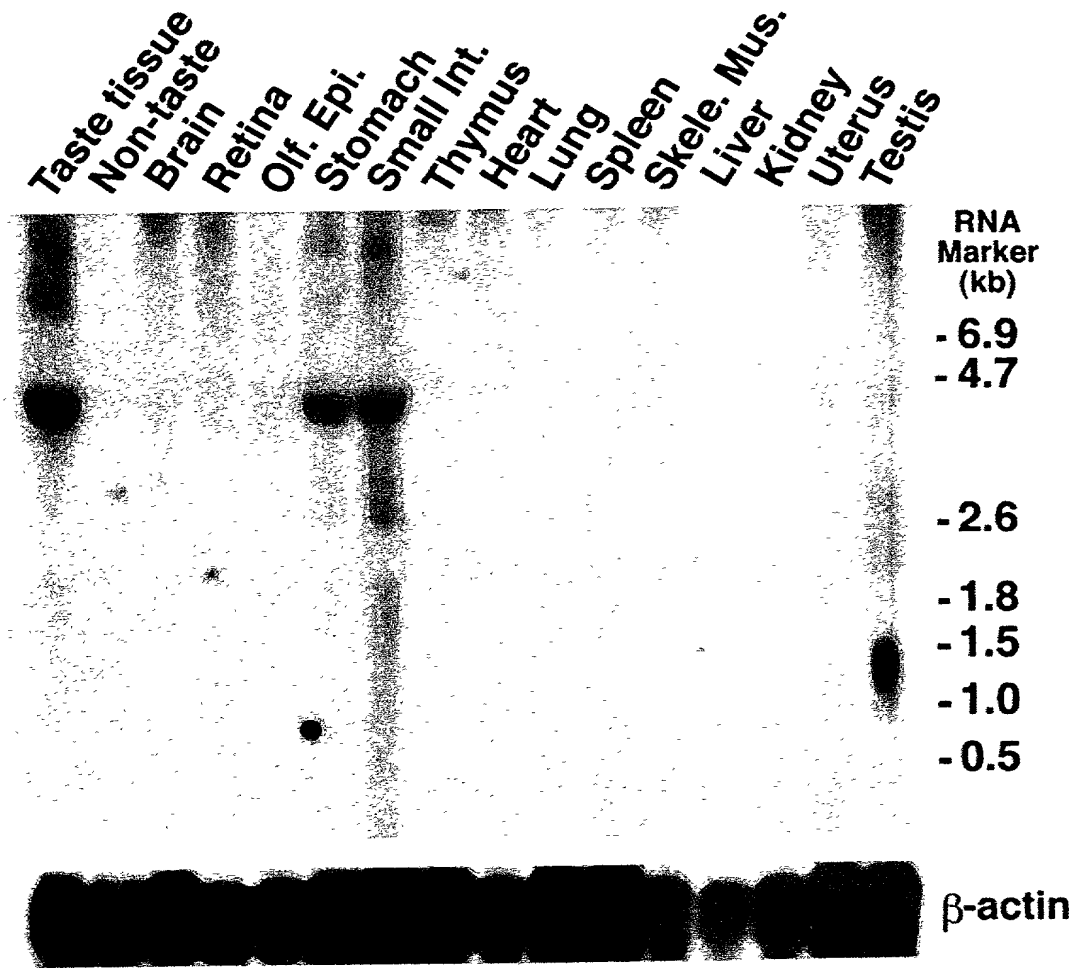
Taste tissue
Non-taste
Brain
Retina
Olf. Epi.
Stomach
Small Int.
Thymus
Heart
Lung
Spleen
Skele. Mus.
Liver
Kidney
Uterus
Testis
RNA Marker (kb)
- 6.9
- 4.7
- 2.6
- 1.8
- 1.5
- 1.0
- 0.5
β-actin

FIG. 7

FIG.8A

FIG.8E

FIG.8B

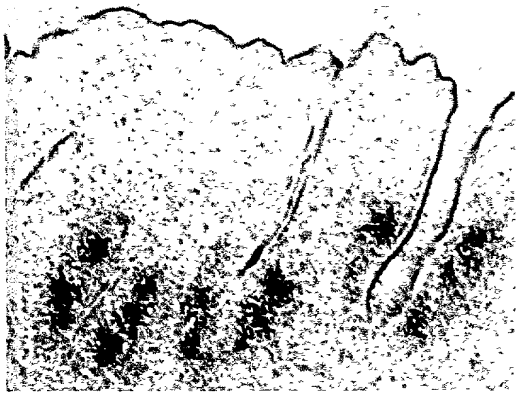

FIG.8C

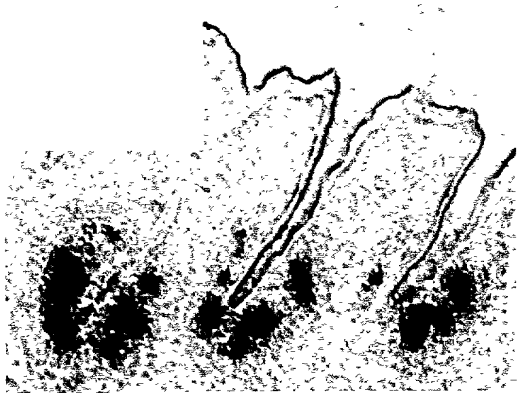

FIG.8D

FIG.9A

FIG.9B

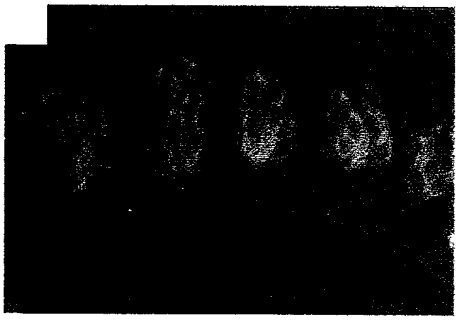

FIG.9C

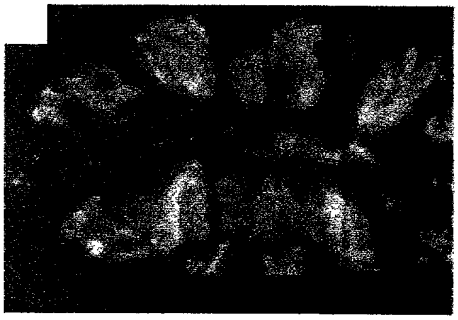

FIG.9D

FIG.9E

FIG.9F

FIG.9G

FIG.9H

FIG.9I

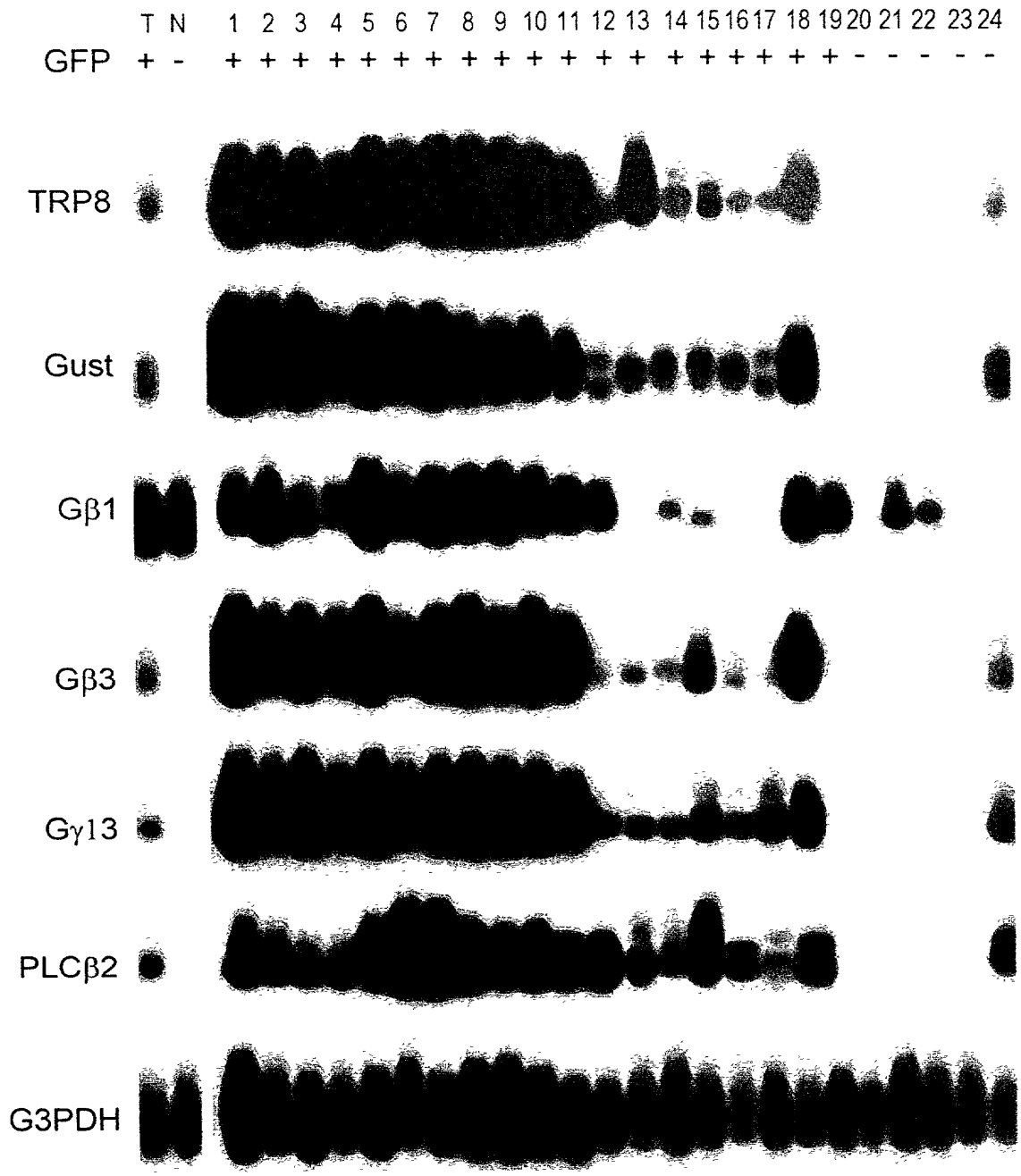
T N 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
GFP + - + + + + + + + + + + + + + + + + + - - - - -
TRP8
Gust
Gβ1
Gβ3
Gγ13
PLCβ2
G3PDH

FIG.10

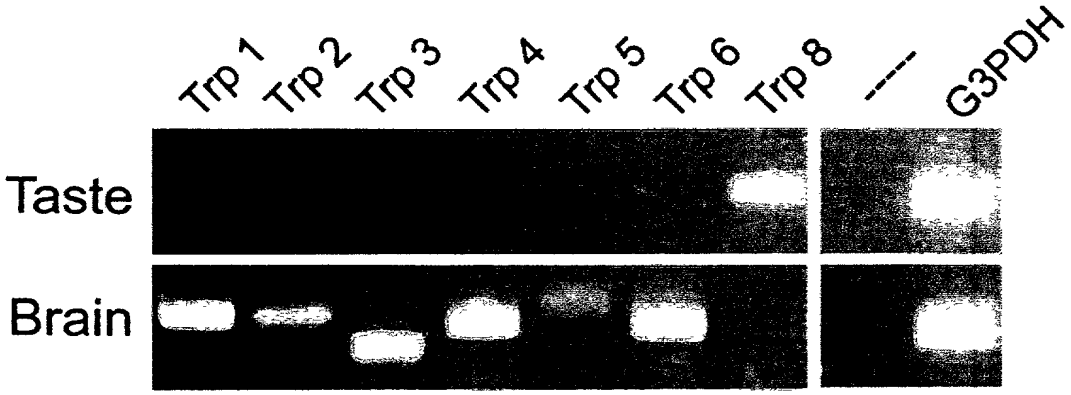

FIG. 11

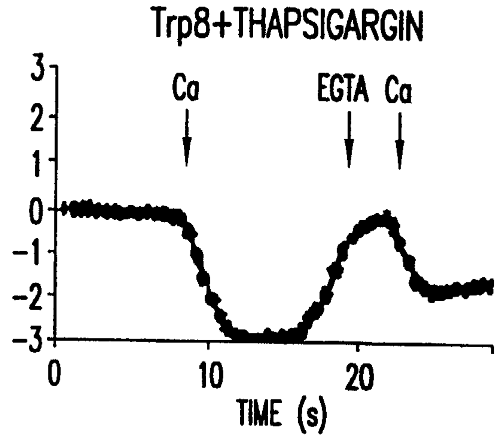

FIG. 12A

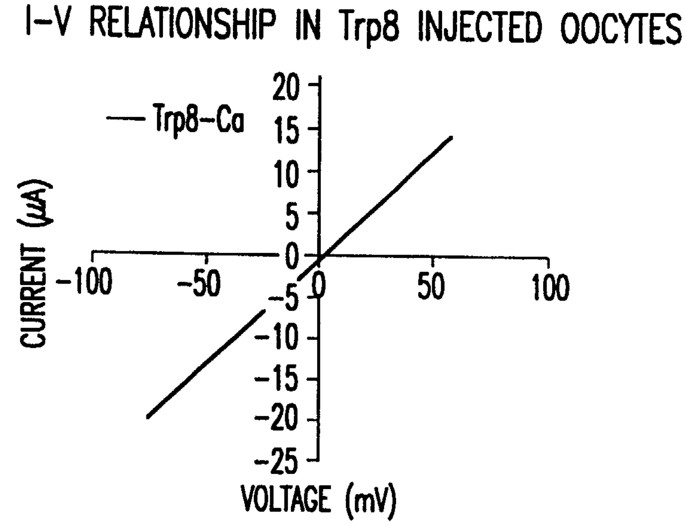

FIG. 12B

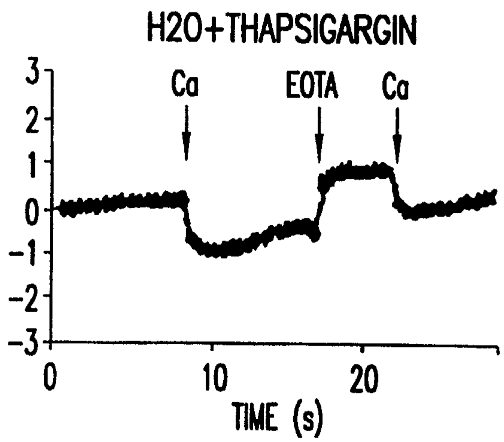

FIG. 12C

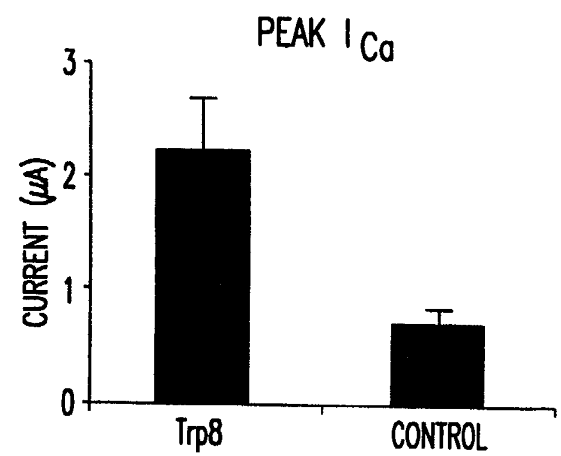

FIG. 12D

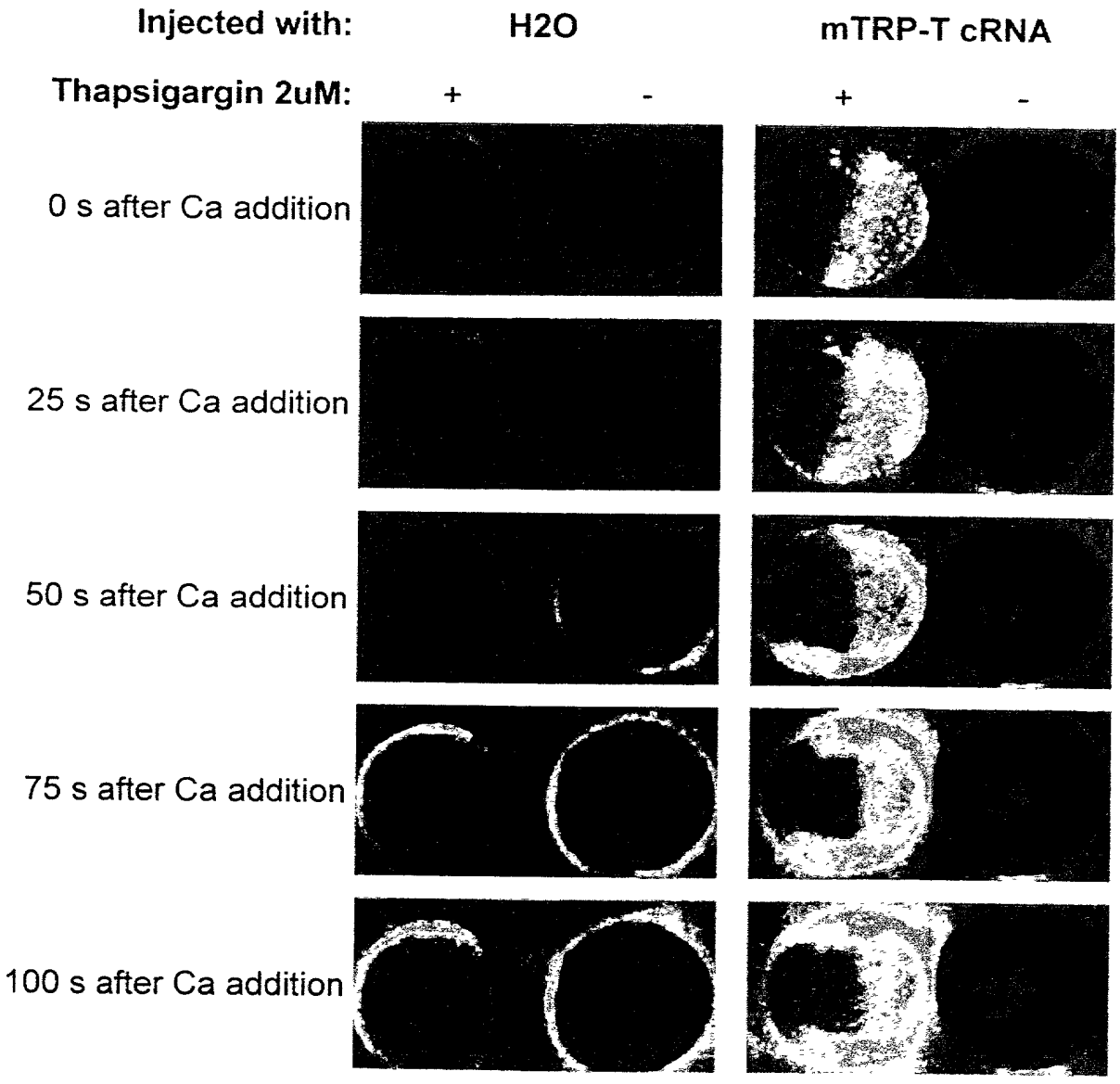
Injected with:
H2O
mTRP-T cRNA
Thapsigargin 2uM:
+
-
+
-
0 s after Ca addition
25 s after Ca addition
50 s after Ca addition
75 s after Ca addition
100 s after Ca addition

FIG.13

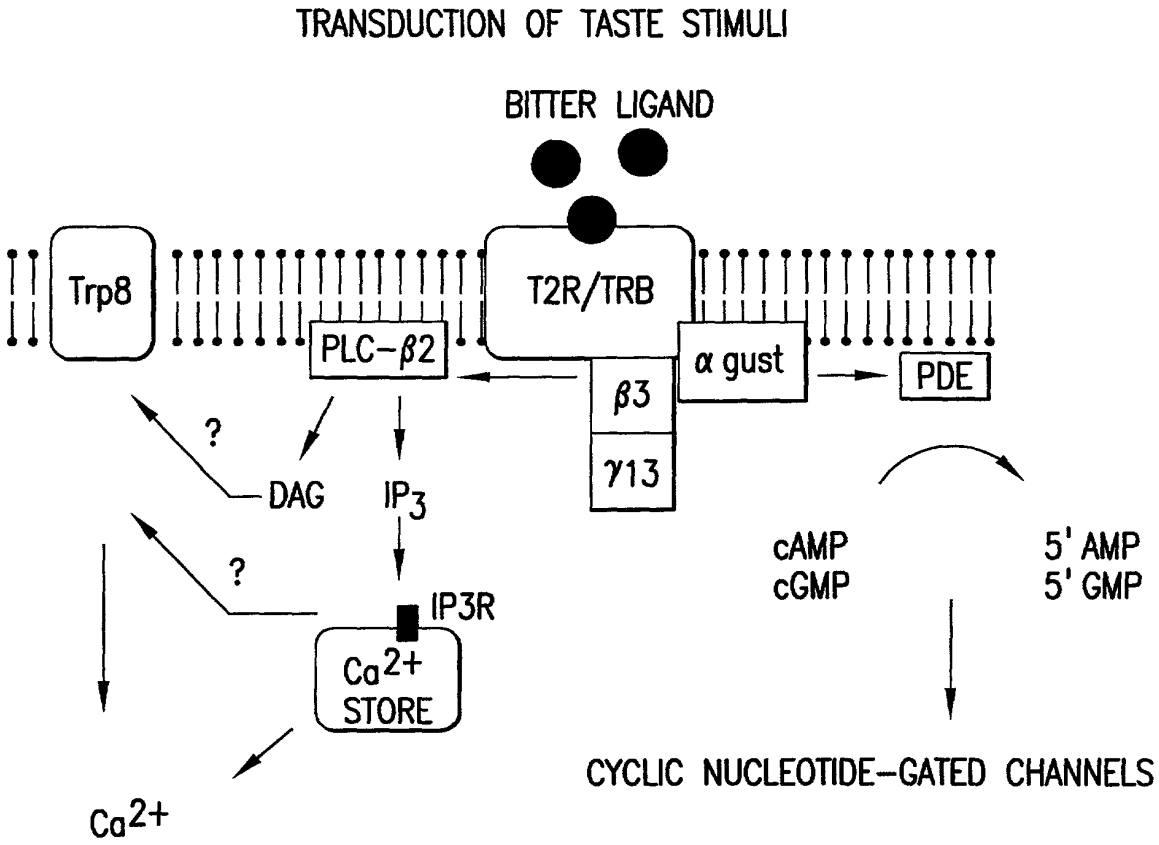
TRANSDUCTION OF TASTE STIMULI
BITTER LIGAND
Trp8
T2R/TRB
PLC-β2
α gust
PDE
β3
γ13
?
DAG
IP3
?
IP3R
Ca2+
STORE
cAMP
cGMP
5' AMP
5' GMP
CYCLIC NUCLEOTIDE-GATED CHANNELS
Ca2+

FIG. 14